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Rapid Report

A novel polymorphism in the human acid sphingomyelinase gene due to size variation of the signal peptide region

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Abstract

Acid sphingomyelinase (ASM) is the lysosomal enzyme required to hydrolyze sphingomyelin into ceramide and phosphocholine. In man, a deficiency of this enzymatic activity leads to Types A and B Niemann-Pick disease (NPD), a panethnic disease with a relatively high incidence among Ashkenazi Jewish individuals. Analysis of the ASM cDNA and genomic sequences revealed a unique hexanucleotide sequence, CTGG(TC)(GT), located within the signal peptide region of the ASM polypeptide (corresponding to the hydrophobic amino acid sequence LVLALALALALA). Notably, five hexanucleotide repeat units were found in the full-length cDNA, while the genomic sequence contained six, suggesting that this region of the ASM gene may be polymorphic. PCR primers were designed to amplify the repeat region and over 700 normal and NPD ASM alleles were analyzed among Ashkenazi Jewish and non-Jewish populations. Five alleles were identified corresponding to nine, seven, six, five and four hexanucleotide repeats, respectively. The allele frequencies were similar among Jewish and non-Jewish populations and no differences were found among normal individuals and Type A and B NPD patients. Thus, it does not appear to be a common cause of NPD. This intriguing repeat polymorphism should be extremely useful to researchers interested in gene identification and characterization of the chromosomal region 11p15.1–p15.4, as well as individuals interested in the biology of this important lysosomal hydrolase.

Keywords: Acid sphingomyelinase; Signal peptide; Repeat polymorphism

Acid sphingomyelinase (sphingomyelin phosphodiesterase; EC 3.1.4.12; ASM) is the lysosomal enzyme required to hydrolyze sphingomyelin into ceramide and phosphocholine [1]. In man, a deficiency of this enzymatic activity leads to the Type A and B forms of Niemann-Pick disease (NPD) [2]. Type A NPD is a rapidly progressive neurodegenerative disorder that generally leads to death by 3 years of age. In contrast, patients with Type B NPD have little or no neurologic involvement and may survive into late adolescence or adulthood. Types A and B NPD have been identified in many different ethnic populations, however they occur at higher frequencies among individuals of Ashkenazi Jewish and North African ancestries [3,4].

The full-length cDNA and genomic sequences encoding ASM have been isolated and the ASM gene has been localized to the chromosomal region 11p15.1–p15.4 [5–7]. The full-length cDNA had an 87 bp 5′ untranslated region, an 1890 bp open reading frame encoding 629 amino acids, and a 370 bp 3′ untranslated sequence. Notably, within the putative ASM signal peptide region a hexanucleotoide sequence, CTGG(TC)(GT), was repeated six times. The corresponding amino acid sequence encoded by this region was LVLALALALALA. Intriguingly, when the ASM genomic region was sequenced and compared to the full-length cDNA [6], only five hexanucleotide repeat units were found, suggesting that this region may be polymorphic. Thus, to investigate the nature of this polymorphism and determine whether allelic expansion was an underlying cause of NPD, the ASM repeat region was analyzed from normal and NPD individuals.

For analysis of the ASM repeat polymorphism, genomic DNA was isolated from cultured cells (fibroblasts or lymphoblasts) or from mixed lymphocytes obtained from whole blood [8]. To isolate genomic DNA, cell pellets were prepared and resuspended in 5 ml of TNE buffer (10 mM Tris-HCl, pH 7.5, 0.1 M NaCl and 1 mM EDTA) containing Proteinase K (final concentration 100 μg/ml) and SDS (final concentration 0.5%). After incubating for 4 h at

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ATG CCC CGC TAC GGA GCG TCA CTC CGC CAG AGC TGC CCC AGG TCC GGC CGG GAG CAG GGA   60
 M   P   R   Y   G   A   S   L   R   Q   S   C   P   R   S   G   R   E   Q   G    20
                                          PCR Sense Primer
CAA GAC GGG ACC GCC GGA GCC CCC GGA CTC CTT TGG ATG GGC CTG GTG CTG GCG CTG GCG  120
 Q   D   G   T   A   G   A   P   G   L   L   W   M   G   L   V   L   A   L   A    80
                                                  ↓
CTG GCG CTG GCG CTG GCT CTG TCT GAC TCT CGG GTT CTC TGG GCT CCG GCA GAG GCT CAC  180
 L   A   L   A   L   A   L   S   D   S   R   V   L   W   A   P   A   E   A   H    60
CCT CTT TCT CCC CAA GGC CAT CCT GCC AGG TTA CAT CGC ATA GTG CCC CGG CTC CGA GAT  240
 P   L   S   P   Q   G   H   P   A   R   L   H   R   I   V   P   R   L   R   D    80
GTC TTT GGG TGG GGG AAC CTC ACC TGC CCA ATC TCG AAA GGT CTA TTC ACC GCC ATC ACC  300
 V   F   G   W   G   N   L   T   C   P   I   C   K   G   L   F   T   A   I   N   100
                                  PCR Antisense Primer
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Fig. 1. ASM cDNA region containing the hexanucleotide repeat polymorphism. 300 bp of the ASM cDNA sequence are shown. The hexanucleotide repeat region is underlined and the putative ASM signal peptide cleavage site is indicated by the arrow. The location of the PCR primers used to amplify this genomic region are also shown.

55° C, genomic DNA was extracted with phenol/chloroform (1:1) and then precipitated with ethanol at −20° C.

For PCR amplification of the repeat polymorphism, sense (5′-GGACTCCTTTGGATGGGCCT-3′) and antisense (5′-CCTTTCGAGATTGGGCAGG-3′) primers were constructed to amplify a genomic region containing the hexanucleotide sequence. The size of this genomic fragment was predicted to be 191 bp based on the previously reported genomic sequence [6], which contained five repeat units. Each 50 μl PCR mixture contained 1 μg of genomic DNA, 1.2 μl of 25 mM Mg_2Cl, 5 μl of PCR buffer (Promega), 2.5 units of *Taq* polymerase (Promega), and 50 μCi of [^{35}S]dATP (Dupont). PCR (30 s each of denaturation (94° C), annealing (62° C) and extension (72° C)) was performed for 30 cycles. The reactions were stopped by the addition of 25 μl of stop solution (95%

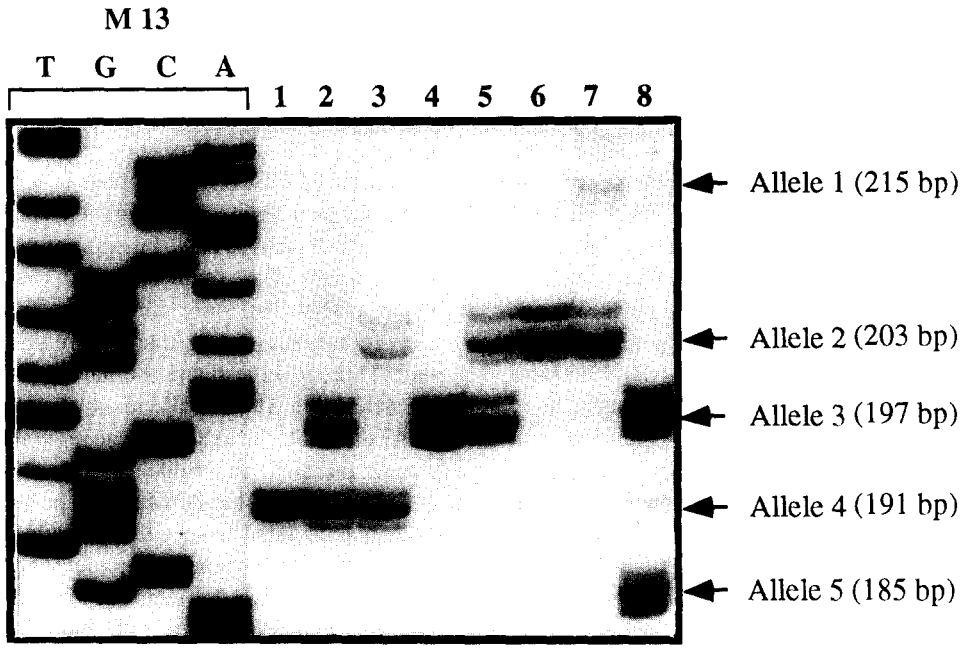

Fig. 2. PCR analysis of the ASM repeat polymorphism in normal individuals. Lanes 1–8 show the amplified PCR products from eight unrelated normal individuals. Note the occurrence of five common alleles, ranging in size from 185 to 215 bp. An M13 sequencing ladder is also shown so that the size of each allele can be accurately determined.

formamide containing 0.1% xylene blue and 0.1% bromophenol blue), denatured for 5 min at 94° C and then loaded onto a 5.6% polyacylamide sequencing gel.

Fig. 1 shows the signal peptide coding region of the ASM cDNA [5], the location of the six hexanucleotide repeat sequences, and the position of the PCR primers used to amplify this region from genomic DNA. PCR analysis was performed on over 700 alleles from normal and NPD individuals. Note that five alleles were identified which corresponded to PCR products ranging in size from 185 to 215 nt (Fig. 2). The size difference of each product was a multiple of six, suggesting that a different number of hexanucleotide sequences was present in each allele. To confirm this observation, the PCR products from several individuals were subcloned and sequenced (not shown). These results demonstrated that the five different sized fragments contained nine, seven, six, five and four hexanucleotide repeats, respectively.

Table 1 shows the frequencies of each allele in normal Ashkenazi Jewish and non-Jewish populations. The most common allele contained six hexanucleotide repeat units (allele 3), followed by alleles 4, 2, 5 and 1 (containing five, seven, four and nine repeat units, respectively). The allele frequencies were generally similar among Ashkenazi Jewish and non-Jewish individuals, however, allele 2 was found twice as frequently in the Ashkenazi Jewish population when compared to non-Jewish individuals. This was reflected by the fact that the genotype 2,2, which occurred in only approx. 5.5% of the non-Jewish individuals, was found in approx. 25% of the Ashkenazi Jewish individuals. In contrast, genotype 3,4 accounted for approx. 42% of the non-Jewish individuals, but only approx. 20% of the Ashkenazi Jewish individuals. Somewhat surprisingly, no individuals were identified in either population who contained eight repeat units. Analysis of over 150 Ashkenazi Jewish and non-Jewish Type A or B NPD patients (346 ASM alleles) demonstrated that the allele frequencies were similar to those found in the normal populations (not shown), suggesting that allele expansion is not a common cause of NPD. To analyze the stability and inheritance of the hexanucleotide repeat polymorphism, several multigeneration families were studied. In all cases, the polymorphic alleles were inherited in a co-dominant manner throughout several generations (Fig. 3).

Thus, the present study was undertaken to determine whether the number of hexanucleotide repeats varied in normal populations and if amplification of this sequence was a possible mechanism underlying NPD. Indeed, five alleles were identified in normal populations, each differing in the number of hexanucleotide repeat units. Alleles

Table 1
Frequency of the ASM repeat polymorphism among normal individuals

	Non-Jewish individuals	Ashkenazi Jewish individuals	*P* value
Allele	($n = 218$) (%)	($n = 268$) (%)	
1	1 (0.50)	0 (0.00)	-
2	27 (12.4)	75 (28.0)	< 0.01
3	110 (50.4)	112 (41.8)	< 0.01
4	76 (34.9)	81 (30.2)	0.0561
5	4 (1.80)	0 (0.00)	-
Genotype	($n = 109$) (%)	($n = 134$) (%)	
1,3	1 (0.90)	0 (0.00)	-
2,2	6 (5.50)	34 (25.4)	< 0.01
2,3	11 (10.1)	7 (5.20)	0.0197
2,3	4 (3.70)	0 (0.00)	-
3,3	24 (22.0)	39 (29.1)	0.0336
3,4	46 (42.2)	27 (20.1)	< 0.01
3,5	0 (0.00)	0 (0.00)	-
4,4	13 (11.9)	27 (20.1)	< 0.01
4,5	4 (3.70)	0 (0.00)	-

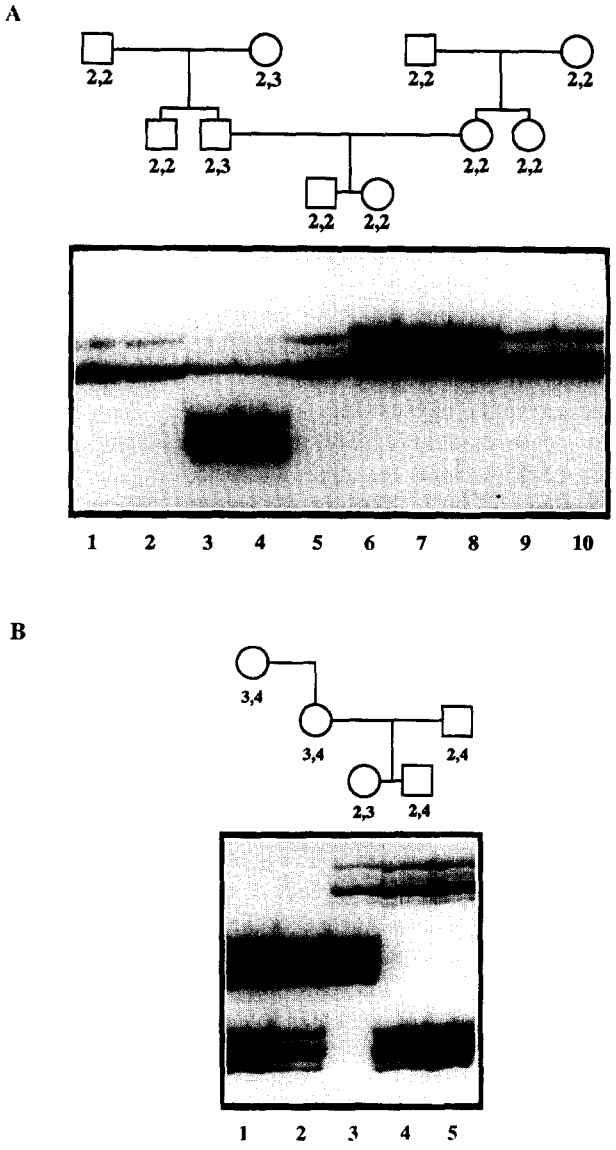

Fig. 3. Inheritance of the ASM polymorphic region in two multigeneration families. Figs. 3A and 3B show two unrelated three generation families. Note that the repeat polymorphism segregated as a co-dominant trait.

1–5 contained nine, seven, six, five and four repeat units, respectively, and occurred in similar frequencies among Ashkenazi Jewish and non-Jewish individuals. The allele frequencies among Ashkenazi Jewish individuals were generally similar, although allele 2 was more commonly found than in non-Jewish populations. The allele frequencies were also similar among normal individuals and Type A and B NPD patients, indicating that amplification of this region is not a common cause of NPD.

It is intriguing that the repeated sequence occurred within the putative ASM signal peptide, which contained a unique hydrophobic core comprised of leucine/alanine or leucine/valine repeats (Fig. 1). Although variations within this region should not affect the catalytic activity of ASM, they could account for abnormal processing, intracellular targeting and/or stability of the enzyme. However, since normal individuals were identified who were homozygous for alleles containing five to seven repeat units, it may be predicted that the size of the signal peptide hydrophobic core may vary from ten to fourteen amino acids without affecting these properties. Notably, the largest and smallest alleles (alleles one and five, corresponding to signal peptide cores of eighteen and eight amino acids, respectively) were only found in combination with one of the other alleles.

Thus, this manuscript reports the occurrence of a novel polymorphism within the ASM gene due to variations in the number of repeated CTGG(TC)(GT) sequences. Since this hexanucleotide sequence occurs within the region of the ASM gene encoding the putative ASM signal peptide, the predicted size of the ASM signal peptide hydrophobic core should vary from eighteen to eight amino acids within normal populations (corresponding to alleles 1–5, respectively). Variations within this region can be easily detected by PCR amplification and should be of value to researchers studying the genomic region 11p15.1–p15.4.

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References

[1] Kanfer, J.N., Young, O.M., Shapiro, D. and Brady, R.O. (1966) Proc. Natl. Acad. Sci. USA 55, 366–369.
[2] Spence, M.W. and Callahan, J.W. (1989) in The Metabolic Basis of Inherited Disease (Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., eds.), pp. 1655–1676, McGraw-Hill, New York, 6th Ed.
[3] Goodman, R.M. (1979) Genetic Disorders Among the Jewish People. Johns Hopkins University Press.
[4] Vanier, M.T., Ferlinz, K., Rousson, R., Duthel, S., Louisot, P., Sandhoff, K. and Suzuki, K. (1993) Deletion of arginine (608) in acid sphingomyelinase is the prevalent mutation among Niemann-Pick disease type B patients from northern Africa. Hum. Genet. 92, 325–330.
[5] Schuchman, E.H., Suchi, M., Takahashi, T., Sandhoff, K. and Desnick, R.J. (1991) Human acid sphingomyelinase. Isolation, nucleotide sequence and expression of the full-length and alternatively spliced RNAs. J. Biol. Chem. 66, 8531–8539.
[6] Schuchman, E.H., Levran, O. and Desnick, R.J. (1992) Structural organization and complete nucleotide sequence of the gene encoding human acid sphingomyelinase. Genomics 12, 197–205.
[7] Pereira, L., Desnick, R.J., Adler, D., Disteche, C.M. and Schuchman, E.H. (1991) Regional assignment of the human acid sphingomyelinase gene by PCR analysis of somatic cell hybrids and in situ hybridization to 11p15.1–p15.4. Genomics 9, 229–234.
[8] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor.